Received: 23 May 2018 | Revised: 12 July 2018 | Accepted: 12 July 2018

DOI: 10.1002/rmv.2006

REVIEW

WILEY

Simplified approach to uncertainty of measurement in the clinical virology laboratory

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Summary

Medical laboratories are required to ensure the quality of their diagnostic results. Quality assurance procedures include quality assessments (internal and external), quality controls (negative, positive, or internal controls), equipment monitoring, and audits. Quality control data may be used to evaluate the uncertainty of measurement. All clinical virology laboratories require a standard operating procedure detailing their consideration of uncertainty of measurement, as this parameter may impact on the overall quality of diagnostic results as well as the clinical interpretation thereof. This review aims to provide a simplified approach to the concept of uncertainty of measurement, specific for clinical virology laboratories.

KEYWORDS

imprecision, quality assurance, statistical analysis, uncertainty of measurement

1 | INTRODUCTION

Laboratories are responsible for ensuring that test results are fit for their clinical purpose by ensuring the quality of their analytical methods.[1] Before any test can be implemented by a diagnostic laboratory, experiments are performed to either validate or verify the assay performance characteristics and to determine if the test is fit for purpose.[2] This forms the initial basis for ensuring quality but does not exempt laboratories from continuous, rigorous, quality assurance activities.

Quality assurance procedures include quality assessments (external or internal), quality controls (QC) (negative, positive, internal controls), equipment monitoring, and audits.[3-5] There are various methods for monitoring QC values which may aid in the evaluation of uncertainty of test results. The type of evaluation done will depend on whether the test provides a quantitative or qualitative result.

The uncertainty of measurement (UOM) is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand (Table 1),[1,6] thereby attempting to quantify the doubt that inevitably exists when any measurement is made.[7] Uncertainty of measurement can be calculated for quantitative tests (such as viral load assays) or qualitative tests that employ ordinal variables and semi-quantitative data (such as serologic assays performed on automated analyzers which may use S/CO [sample over cutoff] values to determine reactivity based on a threshold value). For many qualitative tests in clinical virology, it is not possible to calculate UOM, including tests such as conventional qualitative PCR with end-point detection, rapid lateral flow assays, and immunofluorescence assays. However, laboratories should explain their process of consideration of uncertainty, including the sources of uncertainty for any given assay (Table 2).

There are numerous publications detailing the concept of UOM in medical laboratories. However, not all of these concepts are directly applicable to clinical virology, where a simplified approach may be more appropriate. The statistical analysis of UOM can be complicated and can be presented in different ways.[7,8] Various international metrological and standards bodies jointly developed a Guide to the Expression of Uncertainty in Measurement (GUM) to provide laboratories with a framework of formal metrological terminology and methodology for expressing UOM,[1,9] which is widely used in chemistry and physics but is rarely used in other medical laboratories.[8]

All clinical virology laboratories require a standard operating procedure (SOP) detailing their consideration of UOM, which is required by laboratory accreditation authorities,[6,10] and is not to be confused

Abbreviations: CV, Coefficient of variation; GUM, Guide to the Expression of Uncertainty in Measurement; QC, Quality control; SD, Standard deviation; SOP, Standard operating procedure; UOM, Uncertainty of measurement

TABLE 1 Definitions of commonly used terms in the field of measurement uncertainty

Bias	Numerical difference between the mean of a set or replicate measurements and the true value. This difference (positive or negative) may be expressed in the units in which the quantity is measured or as a percentage of the true value.
Coefficient of variation (CV)	The ratio of the standard deviation to the mean. The higher the coefficient of variation, the greater the level of dispersion around the mean. It is generally expressed as a percentage.
Mean	The arithmetic average of a set of data points.
Measurand	The quantity subject to measurement.
Metrology	Field of knowledge concerned with measurement.
Precision	Closeness of agreement between quantity values obtained by replicate measurements of a quantity, under specified conditions.
Standard deviation (SD)	The standard deviation (SD) is a measure of the distribution of data points above and below the mean. It is used to set acceptable limits for values obtained with QC samples.
Uncertainty of measurement (UOM)	A parameter associated with the result of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand.

TABLE 2 Examples of sources of uncertainty of measurement

Pre-Analytical	Analytical[a]	Post-Analytical
Sampling	Calibrators	Laboratory information system (LIS) issues
Sample preparation	Reagent variation (eg, lot changes)	Incorrect result or value reported
Sample portion selection	Reference materials	
Condition of sample	Input quantities	
Sample transportation and storage	Equipment used	
	Environmental conditions	
	Change in operator	

[a]These uncertainty components form part of the actual test procedure. Uncertainty emanating from these sources is what is routinely measured.

with precision and accuracy determination during validation/verification of new methods. This SOP would therefore form part of the ongoing monitoring of measurement uncertainty.

This review aims to provide a basic overview of UOM, covering both quantitative and qualitative assays, with the goal of presenting a practical, simplified approach for clinical virology laboratories.

2 | UOM: QUANTITATIVE ASSAYS

Estimation of uncertainty may involve calculation of total uncertainty (which would include pre-analytical and post-analytical sources of error), or estimating uncertainty of the testing procedure itself, with the latter being more practical, since laboratories often do not have control of pre-analytical factors.[1] Uncertainty can also be demonstrated by calculating imprecision of long-term QC values and has long been used as a basic quantitative estimate of the confidence that can be placed in a result (Figure 1).[1] Using data from external quality assurance programs is not recommended, because generally far fewer data points are available on which to base the uncertainty estimate.[1] Depending on the range of the reportable values and clinical use of

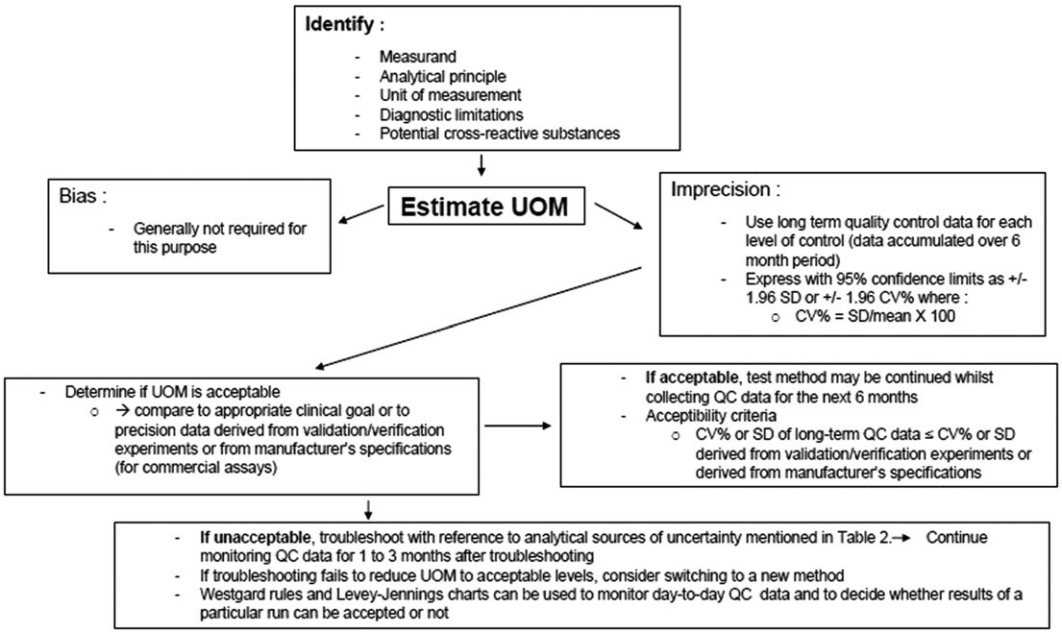

FIGURE 1 Simplified approach to UOM for quantitative clinical virology tests

the test, it may be appropriate to record the estimate of UOM at more than one level of control (for example, a high positive and low positive control).[1] For well-established methods, it is recommended that a minimum of 6 months of QC data be used to calculate routine imprecision, updated twice a year where possible, whereas for new methods, validation or verification data provides an interim estimate of UOM[1] Laboratories may decide to do detailed imprecision calculations[1,10] (usually more suited to clinical chemistry) or basic analysis using the coefficient of variation (CV) only, which may be more suited to clinical virology laboratories (Figure 1). Analytical goals for monitoring UOM will vary and will depend on the type of assay as well as its clinical purpose. If the calculated UOM is appropriate for the clinical goal (for example, an HIV viral load assay with good precision and therefore less uncertainty around the 1000 cp/mL level, which is the clinical cut-off for treatment failure), or the precision data compares favorably

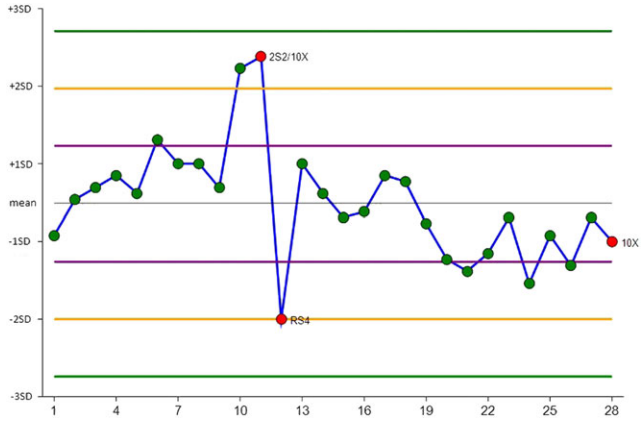

FIGURE 3 Example of a Levey-Jennings chart. The red points are examples of rule violations

Precision analytical goals

Optimum: $CV_A = < 0.25 \times CV_I$

Desirable: $CV_A = < 0.50 \times CV_I$

Minimum: $CV_A = < 0.75 \times CV_I$

Where:

CV_A = Coefficient of variation (analytical), derived from long-term imprecision. The level(s) selected should be close to clinical decision points wherever possible

CV_I = Coefficient of variation (intra-individual), derived from the intra-individual biological variation of the specified measurand (analyte). This may not be available for virological assays.

Bias analytical goals

Optimum: $B_A = < 0.125 (CV_I^2 + CV_G^2)^{1/2}$

Desirable: $B_A = < 0.250 (CV_I^2 + CV_G^2)^{1/2}$

Minimum: $B_A = < 0.375 (CV_I^2 + CV_G^2)^{1/2}$

Where:

B_A = Bias (accuracy, systematic variation)

CV_I = Coefficient of variation (intra-individual), derived from the intra-individual biological variation of the specified measurand (analyte).

CV_G = CV of between - subject (inter-individual) biological variation.

Total error allowable

For methods where bias and imprecision must both meet performance criteria for clinical applications, the two parameters are conveniently combined as Total Error Allowable (Te_a), for which various levels of analytical goal may be set:

Optimum: $Te_a = < 1.65 (0.25\ CV_I) + 0.125 (CV_I^2 + CV_G^2)^{1/2}$

Desirable: $Te_a = < 1.65 (0.50\ CV_I) + 0.250 (CV_I^2 + CV_G^2)^{1/2}$

Minimum: $Te_a = < 1.65 (0.75\ CV_I) + 0.375 (CV_I^2 + CV_G^2)^{1/2}$

Where:

Te_a = Total error allowable

CV_I = Coefficient of variation (intra-individual), derived from the intra-individual biological variation of the specified measurand (analyte).

CV_G = CV of between - subject (inter-individual) biological variation.

FIGURE 2 Alternative analytical goals for monitoring UOM of quantitative tests

TABLE 3 The six commonly used Westgard rules[10]

A	12SD	This rule is used as a warning rule to trigger careful inspection of the control data. If one control measure exceeds the mean ± 2SD, control values in the previous run should be considered to rule out a trend.
B	22SD	This rule detects systematic errors and is violated when two consecutive control values (on the same side of the mean) exceed the same mean + 2SD or mean -2SD limit.
C	41SD	This rule detects systematic error. The rule is violated when four consecutive values exceed the same mean + 1SD or mean − 1SD limit. The run does not need to be rejected if this rule is violated but should trigger recalibration or equipment maintenance.
D	13SD	This control rule detects random error. Violation of this rule may also point to systematic error. The assay run is considered to be out of control when one control value exceeds the mean ± 3SD.
E	R4SD	This is a range rule which detects random error only. This rule is applied only within the current run. The rule is violated when one control measurement in a group exceeds the mean + 2SD and another exceeds the mean − 2SD.
F	10×	This rule detects systematic error, and it is violated when 10 consecutive values fall on the same side of the mean. Its violation often indicates the deterioration of assay reagents. The 10× rule is usually applied across runs and often across materials.

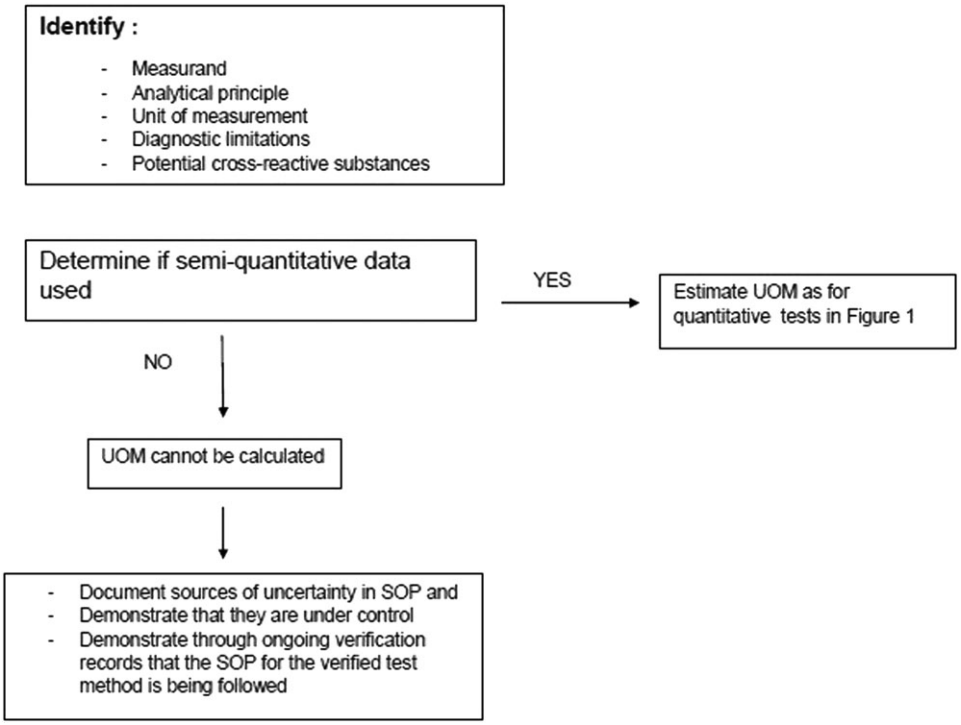

FIGURE 4 Simplified approach to UOM for qualitative clinical virology tests

with data derived from validation/verification experiments or with the manufacturers' specifications (for commercial assays), then the UOM is acceptable. However, more detailed analytical goals may be used (Figure 2)[1], as is often the case in clinical chemistry laboratories. It would be at the discretion of the laboratory director whether or not to use the detailed analytical goals shown in Figure 2. One potential area for applying detailed analytical goals would be for detailed monitoring of UOM in the first few months after a new assay is introduced. For clinical virology, however, we advocate the simplified approach, since new assays are already subjected to rigorous validation/verification experiments.

Analytical bias should not affect measurement uncertainty additional to the imprecision of the method when the test results are clinically interpreted by comparison with reference or previous values produced by the same analytical method.[1] Therefore most clinical virology laboratories do not need to calculate bias as part of ongoing monitoring of UOM, which would have been calculated during validation/verification experiments. Figure 1 illustrates a simplified approach to UOM in clinical virology.

Another commonly used method for monitoring the testing process is by application of the Westgard rules.[11] These are a set of six statistical rules which can be used individually or in combination to detect both random and systematic errors (Table 3). The mean, CV, and standard deviation (SD) are calculated from results obtained after testing the control material on at least 20 separate occasions. The values obtained can then be plotted on a control chart, such as the Levey-Jennings chart (Figure 3), with the mean and limit values of + and −1SD, + and −2SD and + and −3SD delineated for each control used. The Westgard rules can then be applied, depending on where the control value falls. This allows for day-to-day monitoring of control values and is a means of monitoring UOM, allowing for real-time troubleshooting when the uncertainty or error is unacceptable.

3 | UOM: QUALITATIVE ASSAYS

For certain qualitative virology assays, the nature of the test method may preclude rigorous, metrologically, and statistically valid calculation of UOM (Figure 4).[6,7] In these cases the laboratory should at least identify all potential sources for uncertainty and demonstrate that they are under control. The laboratory may be considered to have satisfied the requirement of monitoring of UOM if it can be demonstrated through its validation and ongoing verification records, that the verified method is being followed according to the SOP.[1]

For qualitative tests that employ ordinal variables and semi-quantitative data, it is possible to calculate the UOM similar to what is described above for quantitative tests.

4 | CONCLUSION

Uncertainty of measurement is an important factor to be considered by medical laboratories wishing to provide reliable results of high quality. For many accrediting agencies, it is mandatory for laboratories to have an SOP detailing consideration of UOM. Much has been written about UOM with detailed and complicated statistical analyses for calculation thereof. Many of the complicated statistical calculations are not always necessary or applicable for clinical virology laboratories. Each laboratory should decide which method for monitoring and calculating UOM is used, based on the type of assay under consideration as well as its clinical purpose.

CONFLICT OF INTEREST

None declared.

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How to cite this article: Newman H, Tshisevhe V. Simplified approach to uncertainty of measurement in the clinical virology laboratory. *Rev Med Virol*. 2018;28:e2006. https://doi.org/10.1002/rmv.2006